

URIC ACID STORAGE AND EXCRETION BY ACCESSORY SEX GLANDS OF MALE COCKROACHES

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Abstract—Large amounts of uric acid are stored in the utriculi majores of the accessory sex glands of males of *Blattella germanica*, *Blattella vaga*, *Onychostylus notulatus*, *Lophoblatta* n. sp., *Xestoblatta immaculata*, *Cariblatta minima*, *Epilampra columbiana*, and *Audreia gatunae*. Most of this uric acid is poured over the spermatophore during copulation. The accessory sex gland complex of male cockroaches serves a reproductive function by secreting substances which form the sperm capsule. But in the above species, the utriculi majores serve as storage excretory organs of uric acid between matings and as active excretory organs during copulation.

Uric acid was not detected in the male reproductive systems of thirty-two other species of Blattaria. The known distribution of uricose glands in the Blattaria indicates that these organs were present in the blattellid-blaberid stock and have been lost in some blattellids and most blaberids.

INTRODUCTION

MALE cockroaches produce spermatophores (ZABINSKI, 1933; QADRI, 1938; GUPTA, 1947; JURECKA, 1950; KHALIFA, 1950; VAN WYK, 1952; ROTH and WILLIS, 1952, 1954; NUTTING, 1953) but virtually nothing is known of the chemistry of the accessory sex glands that form the sperm capsules. Recently, uric acid was identified in the utriculi majores (u.m.) of the accessory sex glands of *Blattella germanica* (L.) (ROTH and DATEO, 1964). In this paper we present the results of a survey for the presence of uricose glands in the reproductive systems of forty species of cockroaches.

MATERIALS AND METHODS

The cockroaches were reared on Purina laboratory chow. Only two of the forty species examined were preserved material. Live specimens of the others were available.

The three chromatographic systems that were used for detecting uric acid are described in ROTH and DATEO (1964). For the initial survey, the entire male accessory gland system (exclusive of the phallic gland) was dissected in distilled water, dried, and then powdered. The samples were macerated in 0.1 N Li_2CO_3 , to dissolve any uric acid present, and centrifuged. Usually 5 μl of supernatant was used to spot the paper and the chromatogram was run in NH_4Cl . If uric acid was indicated by u.v.-absorbing spots, the samples were then run in the

n-propanol: NH_3 , and *n*-butanol: acetic acid systems. If these two systems confirmed the presence of uric acid, the u.m. only were dissected, the material was purified, and i.r. and u.v. identifications were made. In *Cariblatia minima* Hebard, *Epilampra columbiana* Saussure, and *Audreia gatunae* Hebard the uric acid was isolated from the entire accessory gland complex because only one male of each species was available. For analysis of spermatophores, specimens were ground with 0.4 ml of 0.01 N LiOH, and 0.4 ml 6% HClO_4 was added to precipitate protein. A 0.5 ml sample of the supernatant was diluted with 0.25 ml H_2O and 2% HClO_4 to 5 ml and uric acid was determined from the absorption at 284 $\text{m}\mu$.

In estimating the amount of uric acid found in the u.m. (Table 2), live weights of the males were determined individually, and their u.m. were removed and dried over silica gel. For each species the dried glands of all five males were pooled and pulverized and a weighed portion was dissolved in 25 ml of 0.01 N NaOH. The uric acid was estimated by measurement of the absorption at 296 $\text{m}\mu$.

RESULTS

Of forty species examined, uric acid was found in the following eight species: *Blattella germanica*, *Blattella vaga* Hebard, *Onychostylus notulatus* (Stål), *Lophoblatta* n. sp. (from Panama), *Cariblatia minima*, *Xestoblatta immaculata* Hebard, *Epilampra columbiana*, and *Audreia gatunae*. Only uric acid was detected by paper chromatography of the alkaline extracts of the u.m. of *E. columbiana*, *A. gatunae*, and *B. germanica*. Extracts of the glands from the other five species indicated the presence of one to four other substances detectable by u.v. absorbance or fluorescence. These were not further investigated.

Accessory sex glands containing uric acid are readily recognizable (Fig. 1(a)–(e)), and after studying *B. germanica* (ROTH and DATEO, 1964) we could predict, on visual examination alone, the presence or absence of uric acid in the glands of other species. The acid is restricted to the u.m. only. These are greatly elongated and relatively few in number compared with the other tubules making up the gland complex. The number of tubules containing uric acid was only one in *A. gatunae*, about five in *E. columbiana*, four to six in *B. germanica* and *B. vaga*, eight to twelve in *X. immaculata*, about ten in *O. notulatus*, and about fifteen in *Lophoblatta* sp. Their chalk-white appearance and the rapid dispersal of the granules in water (in fresh material) are characteristic.

In colonies of *B. germanica*, *B. vaga*, *O. notulatus*, and *Lophoblatta* sp., we found uric acid on the outer surface of old discarded spermatophores and as pieces of white solid. The latter were similar in appearance to the uric acid sometimes attached to recently mated males of *B. germanica* (ROTH and WILLIS, 1952). One dead male of *O. notulatus* had a clump of uric acid adhering to the subgenital plate. Three dried, discarded spermatophores of *X. immaculata* were negative for uric acid; two of these sperm capsules did not have uric acid adhering to the outer surface and the third had a small clump of uric acid which was removed prior to testing for the acid. Large white masses found adhering to

paper in the *X. immaculata* colony contained uric acid and undoubtedly came from males that had recently mated. Two discarded spermatophores of *B. vaga* from which the uric acid adhering to the outer surfaces was removed, were also negative for uric acid. These results are in agreement with our earlier findings with *B. germanica* (ROTH and DATEO, 1964) that uric acid is present only on the outside of the spermatophore and is not incorporated inside the sperm capsule; if it is, it is present in quantities too small ($<1 \mu\text{g}$) to detect with our methods. The u.m. (terminology as used by BREHM (1880) for *Blatta orientalis*) of species, examined in this study, that do not contain uric acid are not excessively long (even in species in which the u.m. are whitish) and their contents are viscous or rubbery and do not disperse in water. Uric acid was not detected visually or by chromatography in the accessory sex gland complexes of thirty-two other species of Blattaria (Table 1).

TABLE 1—SPECIES OF BLATTARIA WHICH LACK URICOSE GLANDS IN THE MALE REPRODUCTIVE SYSTEM

Family and subfamily*	Species
Blattellidae	
Plectopterinae	<i>Supella supellectilium</i> (Serville)
Blattellinae	<i>Shawella coulomiana</i> (Saussure); <i>Symploce hospes</i> (Perkins)
Ectobiinae	<i>Ectobius pallidus</i> (Olivier)
Blaberidae	
Blaberinae	<i>Archimandrita tessellata</i> Rehn; <i>Blaberus craniifer</i> Burmeister; <i>B. discoidalis</i> Serville; <i>B. giganteus</i> (Linnaeus); <i>Byrsotria fumigata</i> (Guérin); <i>Eublaberus distant</i> (Kirby); <i>E. posticus</i> (Erichson)
Zetoborinae	<i>Phortioeca phoraspoides</i> (Walker)
Epilamprinae	<i>Hyporhynchoda litomorpha</i> Hebard
Oxyhaloinae	<i>Griffiniella larvalis</i> Princis; <i>Gromphadorhina portentosa</i> (Schaum); <i>Leucophaea maderae</i> (Fabricius); <i>Nauphoeta cinerea</i> (Olivier)
Panchlorinae	<i>Capucina patula</i> (Walker); <i>Panchlova nivea</i> (Linnaeus)
Diplopterinae	<i>Diploptera punctata</i> (Eschscholtz)
Pycnoscelinae	<i>Pycnoscelus surinamensis</i> (Linnaeus)
Blattidae	
Lamproblattinae	<i>Lamproblatta albipalpus</i> Hebard
Blattinae	<i>Blatta orientalis</i> Linnaeus; <i>Deropeltis erythrocephala</i> (Fabricius); <i>Periplaneta americana</i> (Linnaeus); <i>P. australasiae</i> (Fabricius); <i>P. fuliginosa</i> (Serville); <i>P. brunnea</i> Burmeister.
Polyzosteriinae	<i>Eurycotis decipiens</i> (Kirby); <i>E. floridana</i> (Walker); <i>Melanozosteria semivitta</i> (Walker); <i>Platyzosteria melanaria</i> (Erichson)

* After MCKITTRICK (1964); and personal communication.

No detectable uric acid was associated with dropped spermatophores of *B. craniifer*, *B. discoidalis*, *C. patula*, *B. fumigata*, *D. punctata*, *E. posticus*, *L. maderae*, and *N. cinerea*, all species in which uric acid was not found in the accessory sex glands.

An estimation of uric acid accumulation in the u.m. of four species of cockroaches is shown in Table 2. In *B. germanica*, *O. notulatus*, *Lophoblatta* sp., and *X. immaculata*, the amounts were 13, 47, 43, and 25 mg/g of live wt., respectively. The males of the four species used for these data were taken from cultures and their sexual histories were unknown. In all specimens, the glands were turgid with uric acid, although not necessarily stretched to their fullest extent (particularly in *B. germanica*). The amount of uric acid in the u.m. depends largely on the frequency of mating since these glands are virtually emptied during copulation (ROTH and WILLIS, 1952). With males of varying sexual activity, one may find considerable variation in the amount of uric acid in the u.m. (Fig. 1(d)). In *O. notulatus*, so much uric acid may be excreted by the male that the female's genital segments gape open and cannot be closed; however, the sperm capsule is completely hidden by the uric acid coating (Fig. 1(f)).

TABLE 2—URIC ACID CONTENT OF THE UTRICULI MAJORES OF MALES OF 4 SPECIES OF COCKROACHES

Live wt. of males (mg)*		Dry wt. of u.m. (mg)		Uric acid in u.m. (mg)	
Range	Mean \pm S. E.†	Range	Mean \pm S. E.	Per g u.m. (dry wt.)	Per g. male (live wt.)
<i>Blattella germanica</i>					
48.9-56.4	51.5 \pm 1.3	0.7-1.0	0.8 \pm 0.05	820	13
<i>Onychostylus notulatus</i>					
42.5-52.1	47.5 \pm 1.8	3.0-3.5	3.3 \pm 0.1	680	47
<i>Lophoblatta</i> sp.					
58.0-90.0	76.1 \pm 6.0	1.9-8.3	5.0 \pm 1.2	660	43
<i>Xestoblatta immaculata</i>					
376.2-438.4	409.1 \pm 9.9	8.8-14.2	11.6 \pm 0.9	880	25

* Based on five males for each species.

† S.E. = Standard error.

DISCUSSION

Uric acid is the characteristic end-product of nitrogen metabolism in terrestrial insects. It may be found in the blood, fat body, and malpighian tubes, and is usually eliminated in urine and faeces (WIGGLESWORTH, 1942; PATTON, 1953). In cockroaches, uric acid accumulates in special cells of the fat body and none seems to be found in the malpighian tubules (KILBY, 1963). Since any method of removing a substance from metabolic activity becomes a process of excretion (CRAIG, 1960), the accessory sex glands of the males of the eight species of cockroaches that contain uric acid are excretory organs. The u.m. may be considered as storage excretory organs of uric acid between matings and as active excretory organs during copulation when the waste product is poured over the spermatophore.

The accumulation of inordinate amounts of uric acid in the fat body may have profound effects on uricotelic insects (MARTIGNONI, 1964). In *B. germanica*,

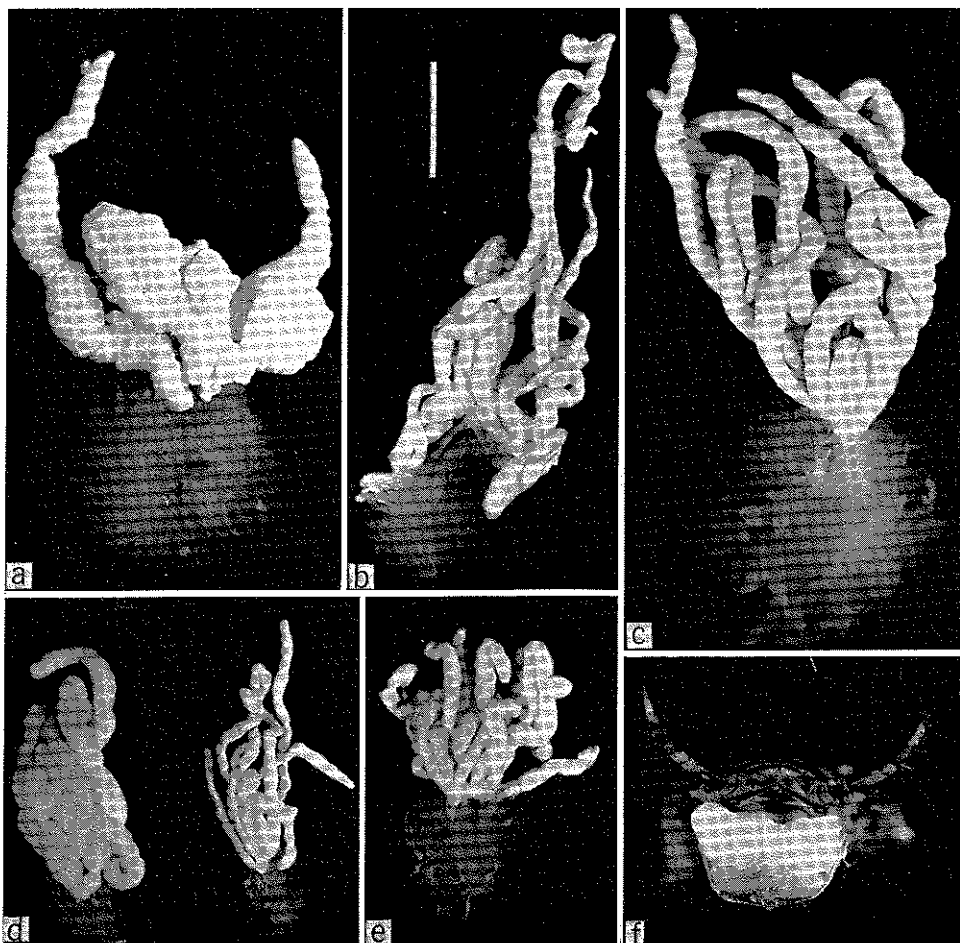


FIG. 1. (a-e) Male accessory sex glands (exclusive of the phallic gland) of cockroaches. The utriculi maiores are greatly elongated and filled with white uric acid. Vertical line = 2 mm (all with equal magnification): (a) *Epilampra columbiana*; (b) *Lophoblatta* sp.; (c) *Xestoblatta immaculata*; (d) *Blattella vaga* (two gland complexes showing different amounts of uric acid); (e) *Onychostylus notulatus*. (f) End view of a recently mated female of *O. notulatus*, showing the large white mass of uric acid which completely covers the spermatophore.

Blatta orientalis, and *P. americana*, excessively high protein diets reduce longevity; in the latter species the fat body becomes greatly enlarged with murexide positive white deposits which are presumably urates of uric acid (HAYDAK, 1953). It is not known if excessive uric acid accumulation in the u.m. is harmful when males are prevented from mating. It should be enlightening to determine the relative importance of uric acid storage and excretion by way of spermatophore formation, as compared with fat body storage of uric acid. Since males can eliminate the uric acid in the u.m. it would appear that this is a more effective excretory device than fat body storage. Although active elimination of uric acid from the u.m. depends on copulation, the gregarious behaviour of cockroaches (ROTH and WILLIS, 1960) ensures that the males will have access to females frequently enough so that mating may be an important excretory function for the male. During periods of low protein intake, uric acid in the fat body diminishes (HAYDAK, 1953) and since degradation of uric acid occurs *in vivo*, breakdown of uric acid is believed to account for part of the uric acid decrease in the fat body (MCENROE, 1956). If, during nutritional stress, degradation products of uric acid are utilized by the cockroach, then storage excretion in the fat body may have some advantage. Because large amounts of uric acid are stored in the reproductive system, *B. germanica* and the other species with uricose glands should make excellent tools for studies involving nitrogen metabolism and uric acid biosynthesis.

We (1964) have been unable to confirm KHALIFA's (1950) statement that material from the u.m. (i.e. uric acid) is present *inside* the spermatophore of *B. germanica* or, in the present study, of *B. vaga* and *X. immaculata*. DAVEY (1958) found that in the hemipteran *Rhodnius prolixus* Stål the sperm migrate from the spermatophore into the female as a result of contractions set up in the oviduct by an opaque secretion from the male's accessory sex gland. He observed that a suspension of the glands of *Periplaneta* activated the oviducts of *Rhodnius* in the same way. This was attributed to the milky granular secretion associated with the spermatophore of '*B. germanica* and *P. americana*' described by KHALIFA (1950). However, Khalifa did not mention a milky granular secretion in *P. americana*. The contents of the u.m. of *B. germanica* differs from that found in *P. americana* and there is no reason to believe that uric acid in the u.m. would activate the female's oviducts.

Spermatophores are a primitive feature in insects (HINTON, 1964) and occur in different orders (KHALIFA, 1949; GHILAROV, 1958, 1959; DAVEY, 1960; ALEXANDER, 1964). That the male accessory sex glands in some species of cockroaches have an excretory function raises certain questions about the evolution of these glands. At one time the accessory sex glands may have had a dual role—reproductive and excretory. The elimination of uric acid during copulation not only rid the insect of an important waste product, but may also have served to protect the spermatophore. The biological use of an excretory product is not unusual among insects (SNODGRASS, 1935).

Of the eight species that had uric acid in the u.m., six belong to two sub-families of Blattellidae. *Lophoblatta* sp., *O. notulatus*, and *Cariblatta minima* are

Plectopterinae, and *B. germanica*, *B. vaga*, and *X. immaculata* belong to the Blattellinae. These species have 4–15 tubules comprising the u.m. Four other species of Blattellidae (Table 1) did not have uricose glands. *Epilampra columbiana* and *Audreia gatunae* belong to the Epilamprinae and were the only members of the nineteen Blaberidae which had uric acid in the accessory sex glands. The u.m. of *A. gatunae* consists of a single tubule. All eleven species of the Blattidae lacked uricose glands (Table 1). At present, the known distribution of uricose glands in the male reproductive system of cockroaches indicates that these glands were present in the blattellid-blaberid stock, and that they have been lost in some blattellids and most blaberids. The single tubule in *A. gatunae* may represent a marked reduction in the uricose glands. In those species in which the uricose glands are absent or have been lost, the excretion of uric acid is done chiefly by the fat body. However, the fat body probably also serves as a storage excretory system in the males that have uricose glands.

We have examined a large number of male orthopteroid insects (Mantidae, Phasmidae, Acrididae, Gryllidae, and Tettigoniidae) and none have uricose glands. Among insects these glands may be unique in some male Blattaria. However, it is interesting that uric acid is present in very small amounts in mammalian semen (SPECTOR, 1956). The hypothesis that the uric acid of the male's accessory sex glands may have served, at one time, to protect the spermatophore from being eaten, will be developed elsewhere in a paper dealing with the evolution of uricose glands.

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